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Expression of C-reactive protein by renal cell carcinomas and unaffected surrounding renal tissue

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Background. Elevation of plasma C-reactive protein (CRP) is common in patients with renal cell carcinoma (RCC). Renal tubular epithelial cells are capable of synthesizing CRP. Although production of interleukin (IL)-6 has been described in RCC, CRP expression by carcinoma cells has yet not been investigated.

Methods. In the present study we analyzed CRP plasma levels as well as intratumoral CRP and IL-6 expression of RCC from 40 patients who underwent radical nephrectomy by means of quantitative real-time polymerase chain reaction (PCR) and immunohistochemistry. For each tumor, specimens were obtained from tumor center, tumor margin, and unaffected surrounding renal tissue.

Results. Preoperative plasma CRP levels correlated significantly with tumor stage ($P = 0.05$) and grade ($P < 0.01$). CRP mRNA expression was detected in 26 of 33 (79%), 30 of 36 (83%), and 32 of 36 (89%) samples from tumor center, tumor margin, and unaffected surrounding tissue, respectively. However, levels of CRP mRNA were significantly higher in tumor tissue compared to adjacent renal tissue ($P < 0.01$). Clear cell carcinoma exhibited significantly higher CRP mRNA levels than papillary carcinoma ($P < 0.05$). CRP plasma levels correlated significantly with quantitative levels of CRP mRNA within tumors ($P < 0.0001$). Immunohistochemically, strong CRP production was observed both in tumor cells and in tubular epithelial cells in unaffected tissue, respectively. All kidneys expressed IL-6 mRNA in the tumor and/or the unaffected tissue, but levels of intratumoral IL-6 mRNA showed no significant correlation with CRP plasma levels or local CRP transcription.

Conclusion. In patients with RCC, a tumor-derived origin of some plasma CRP is likely. Activity of the IL-6/CRP network in RCC contributes to the accumulating evidence of the acute-phase reaction as a local inflammatory process.

Key words: C-reactive protein, renal cell carcinoma, local, acute-phase reaction.

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C-reactive protein (CRP) is an acute phase reactant, whose plasma levels increase up to 1000-fold in response to microbial infection, trauma, infarction, autoimmune, or malignant diseases. Due to its ability to bind to several microbial cell wall and cell membrane components as well as to nuclear antigens, it plays an essential role in the clearance of infective or necrotic particles via phagocytes. Upon ligand binding, CRP is capable of activating the classic pathway of complement, but inhibits the formation of the terminal membrane attack complex. Considering the numerous and sometimes opposing activities of CRP it is regarded as a proinflammatory as well as anti-inflammatory molecule active in innate immunity and tissue homeostasis (for review, see [1]). The origin of plasma CRP was initially described as being restricted to the liver [2]. However, recent data have demonstrated a local expression of this prototypic acute phase protein in the kidney, the respiratory tract, the thymus, adipose tissue, in smooth muscle cells of normal and diseased vessels, and in neuronal cells, rejecting the liver as the only site of CRP production [3–9].

An increase in plasma CRP without apparent inflammatory disease or without any other underlying condition known to be associated with minor CRP elevation (such as atherosclerosis, infarction, obesity) may suggest clinically unapparent malignancy, as nearly every malignant disease induces perturbations of plasma levels of acute phase proteins. Indeed, CRP elevations have been found in the course of all types of carcinomas as well as lymphomas, sarcomas, and cerebral tumors [10, 11]. In renal cell carcinoma (RCC), the rise in plasma CRP correlates with tumor burden and metastases. Therefore, CRP has been identified as one of the most significant predictor of patients' overall survival [12–14]. The origin of "tumor CRP" is unexplained or, at most, linked to interleukin (IL)-6 secretion by the tumor stimulating CRP synthesis in hepatocytes [15, 16]. However, CRP expression by malignant cells itself has not been investigated in detail, explaining tumor-associated CRP elevations more easily.

Table 1. Preoperative and perioperative characteristics of 40 renal cell carcinoma (RCC) patients

Patient	Gender	Age *years*	Histology	Tumor size *cm*	Stage	Grade	White blood cell count/*nL*	Plasma CRP *mg/L*	Urine CRP/MPO μ*g/L*	Urine bacteriology
1	M	64	Clear cell	6.9	T2	G2	6.2	40	0	Negative
2	M	69	Clear cell	10.3	T3	G1	10.3	10	0	Negative
3	M	60	Clear cell	2.5	T1	G2	7.7	18	0	Negative
5	M	65	Clear cell	6.0	T2	G2	8.1	7	0	ND
6	F	86	Clear cell	4.1	T3	G2	11.8	15	0	Negative
7	M	41	Clear cell	7.5	T2	G2	12.6	12	0	Negative
8	M	89	Papillary	1.0	T1	G2	5.9	0	ND	ND
9	F	67	Papillary	2.5	T1	G3	7.9	32	0	ND
10	M	60	Clear cell	6.0	T1	G1	8.2	4	ND	ND
11	M	55	Clear cell	11.0	T3	G2	6.9	0	ND	Negative
12	F	70	Clear cell	6.0	T3	G3	9.8	94	0	Negative
13	M	62	Papillary	7.5	T3	G2	6.0	13	ND	Negative
14	M	80	Clear cell	4.5	T1	G2	7.3	71	ND	ND
15	F	46	Clear cell	5.6	T1	G3	6.7	0	ND	ND
16	M	60	Clear cell	2.8	T1	G2	4.8	0	ND	Negative
17	M	81	Clear cell	12.0	T3	G3	8.0	121	ND	ND
18	F	68	Clear cell	6.0	T2	G2	7.4	0	ND	ND
19	M	64	Clear cell	4.5	T1	G2	8.4	13	ND	ND
20	M	50	Clear cell	3.0	T1	G2	7.0	2	ND	Negative
21	M	71	Clear cell	6.0	T1	G3	8.1	28	ND	ND
22	F	56	Clear cell	2.7	T1	G2	9.1	0	ND	ND
23	M	71	Papillary	4.5	T1	G2	8.1	0	ND	ND
24	M	61	Papillary	5.3	T3	G2	8.6	103	ND	ND
25	M	68	Papillary	5.4	T1	G2	9.5	7	ND	ND
26	F	61	Clear cell	5.5	T1	G2	7.5	3	ND	Negative
27	F	71	Clear cell	13.0	T2	G2	7.9	14	ND	ND
29	M	59	Papillary	2.6	T1	G2	6.1	0	ND	Negative
30	F	54	Papillary	1.8	T1	G2	6.0	0	ND	Negative
31	F	62	Clear cell	8.0	T2	G2	9.2	60	ND	Negative
32	F	61	Clear cell	2.6	T1	G2	7.2	10	ND	Negative
33	M	46	Clear cell	3.6	T1	G2	12.6	0	ND	Negative
34	M	68	Clear cell	6.5	T3	G2	11.4	1	ND	Negative
35	M	63	Clear cell	12.5	T3	G2	6.5	9	ND	ND
36	M	73	Clear cell	13.0	T3	G1	5.1	3	ND	Negative
38	F	77	Papillary	4.0	T1	G2	7.6	8	ND	Negative
39	F	38	Spindle cell	22.0	T3	G2	6.0	0	ND	ND
40	M	76	Clear cell	12.0	T3	G3	6.4	54	ND	ND
41	M	40	Clear cell	7.4	T3	G3	6.5	7	ND	neg.
46	M	63	Clear cell	9.0	T3	G2	7.9	13	ND	neg.
47	M	48	Clear cell	9.0	T3	G2	6.2	9	ND	neg.

Abbreviations are: M, male; F, female; ND, not determined.

Therefore, we investigated production of CRP by RCC cell lines and in tumor-bearing kidneys and compared the results to preoperative CRP plasma levels as well as to tumor stage, grade, and IL-6 mRNA expression of the tumors.

METHODS

Patients' characteristics and tissue collection

Initially, 47 consecutive patients with unilateral renal masses suspicious of RCC by means of computed tomography or magnetic resonance imaging were prospectively enrolled into this study. Seven patients were excluded from further investigations, since the histologic examination revealed tumors different from RCC (two benign tumors and five chronic infections). All patients undergoing radical nephrectomy gave their informed consent for scientific investigations of the removed kidneys. This study was approved by our local ethic committee. Clinical and histologic data for the remaining 40 patients are given in Table 1. To exclude urinary tract infections prior to nephrectomy, 22 patients gave urine preoperatively for bacteriology. None of these patients had a significant bacteriuria (see Table 1 for details). Moreover, white blood cell counts were measured preoperatively in all patients. Leukocytes ranged between 4.8 and 12.6 per nanoliter, median 7.7/nL. Those with counts above 10.0/nL neither had fever nor CRP levels different from the rest of the group. In addition, CRP and myeloperoxidase (MPO) concentrations were determined in preoperative urine samples of eight patients by means of a highly sensitive immunoluminometric assay [17]. This assay proved to be helpful in the diagnosis of urinary tract infections of kidney transplant recipients. As none of these early patients tested positive for urinary CRP or MPO, we did not follow the remaining patients.

For each tumor, specimens were obtained from the tumor center (which was necrotic in many cases), the vital

tumor margin and the unaffected surrounding renal tissue immediately (less than 3 minutes) after nephrectomy. For immunohistochemistry, a piece of about 0.125 cm^3 was cut from each of the three sites, covered with tissue-freezing medium (Jung, Nussloch, Germany) and stored at −80°C. For transcriptional analysis, tissue specimens from each region were obtained with an 18 gauge Trucut biopsy needle. Samples were immediately transferred into a guanidine isothiocyanate–containing lysis buffer substituted with 2-mercaptoethanol (RLT buffer) (Qiagen, Hilden, Germany) and stored at −80°C until further use. The nephrectomy specimen was then fixed in 4.5% buffered formalin and prepared for histopathologic examination. Classification of tumor stage was done according to the Union International Contre le Cancer (UICC) classification [18], and staging according to the classification of Thoenes, Störkel, and Rumpelt [19]. All cases were reviewed for correct stage and grade by a local pathologist, who was blinded to the CRP data. Due to mostly negative or unknown data, we did not include lymph node status (N) and status of metastases (M) into our analysis.

RNA extraction and cDNA synthesis

Samples for RNA extraction were thawed in batches of 12 and processed immediately thereafter. Tissue in lysis buffer was further disrupted by thorough vortexing for about 1 minute. Afterward, lysed tissue was homogenized by spin columns (homogenizer) (Qiagen) and flow-through was loaded onto the silica membranes of RNeasy mini-columns (Qiagen). Further extraction followed the recommendations of the manufacturer. Total RNA was eluted in 30 mL of RE buffer (Qiagen). Twenty microliters of RNA were then reverse transcribed using random primer (Gibco, Karlsruhe, Germany) and Superscript II Reverse Transcriptase (Gibco). The resulting cDNA was stored at −80°C and thawed first for CRP and then for IL-6 mRNA determination.

Quantitative real-time polymerase chain reaction (PCR)

Quantitative real-time PCR (TaqMan™ PCR; Applied Biosystems, Darmstadt, Germany) for CRP mRNA detection was performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems) and exactly followed our recently published protocol [3]. In brief, CRP mRNA was detected in a multiplex real-time PCR reaction amplifying CRP and hypoxanthine phosphoribosyltransferase (HPRT) cDNA within the same tube. HPRT coamplification was used for normalization of the CRP amplification signal avoiding tube-to-tube variations and pipetting errors. All determinations were done in duplicate taking the mean of both reactions for quantitative analysis. Quantification of relative CRP mRNA levels was carried out by calibrating against a cDNA from maximally stimulated Hep3B cells, which was amplified in each experiment in duplicate as well. Results are always given as multiplicities of this calibrator. The heptoma-derived cell line Hep3B exhibits an inducible CRP transcription and has been studied extensively regarding its CRP expression [3, 20]. After stimulation, it is a reliable source of detectable CRP mRNA. Since, to our best knowledge, there is no renal epithelial cell line with a comparable CRP expression, Hep3B cells were stimulated with a conditioned medium at a density of 1×10^6/mL in 10 mL culture flasks for 48 hours prior to RNA extraction for preparation of the calibrator cDNA. Conditioned medium had already been shown previously to yield the highest CRP induction in Hep3B cells after 48 hours [3]. It was made by stimulating peripheral blood mononuclear cells of a healthy donor with lipopolysaccharide (10 ng/mL) over night, using the filtered culture supernatant as conditioned medium. The cDNA from conditioned medium–stimulated Hep3B cells was aliquoted into 10 μL specimens, which were thawed only twice to avoid significant degradation due to repeated freeze-thaw cycles. Only one batch of Hep3B cDNA was used throughout the whole study. IL-6 mRNA was detected by quantitative real-time PCR as well, using predeveloped primer/probes purchased from Applied Biosystems. Forty cycles of amplification were performed at 60°C for 1 minute and at 95°C for 15 seconds. Calibration was carried out with the same calibration sample used for CRP mRNA quantification. Results were again calculated as multiplicities of this calibrator.

Immunohistochemistry

Staining for CRP protein using the APAAP technology followed the protocol described before in detail [3]. Immunohistochemistry was done on fresh-frozen, 4 μm thick sections of the respective tumor/kidney areas or on cell monolayers of the RCC cell line CaKi, which were grown on LabTec™ chamber slides (Nunc, Wiesbaden, Germany). The monoclonal anti-CRP antibody (Sigma Aldrich, Munich, Germany) had already been shown to stain specifically native CRP by preincubation with native recombinant CRP (Calbiochem, San Diego, CA, USA) and subsequent loss of staining as well as by isotype control staining used in each experiment [3].

Statistical analysis

Comparison of CRP mRNA levels between different regions of nephrectomized kidneys was done by nonparametric Wilcoxon test (paired samples). Bivariate correlation analyses were performed by Pearson test. Results were confirmed by partial correlation correcting for age, gender, and histology of the tumor. CRP plasma and expression levels at increasing tumor stages and grades were compared with the nonparametric Kruskal-Wallis test. Values are given as mean ± SD.

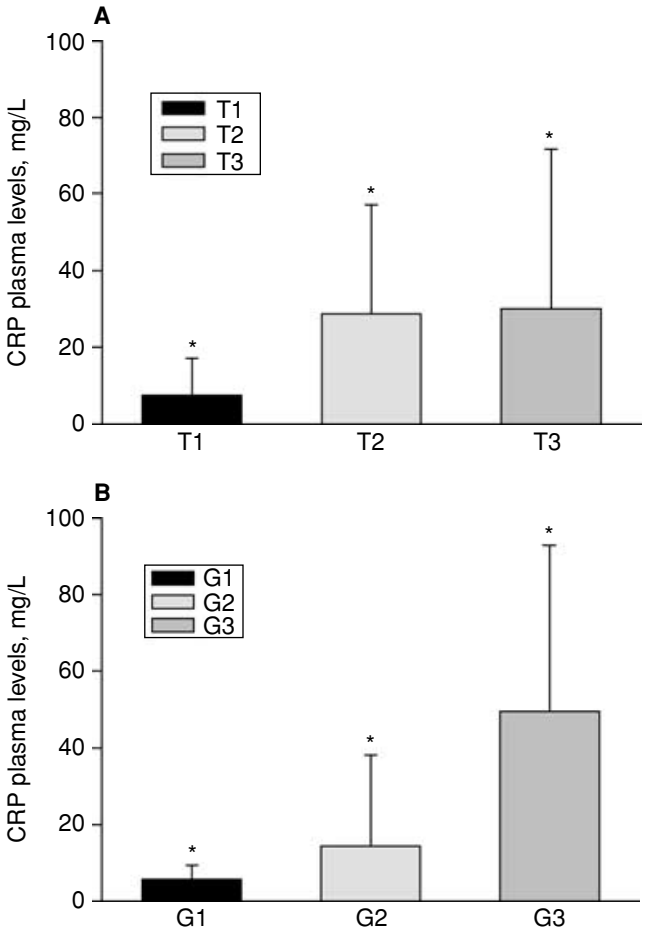

Fig. 1. Preoperative C-reactive protein (CRP) plasma levels of 40 renal cell carcinoma (RCC) patients in correlation to tumor stage and grade. (*A*) Tumor stage according to the Union International Contre le Cancer (UICC) classification from 1997 [18]. *$\chi^2 = 5.68$; $P = 0.059$ by Kruskal-Wallis test. (*B*) Tumor grading according to the classification by Thoenes, Störkel, and Rumpelt from 1986 [19]. * $\chi^2 = 6.52$; $P < 0.05$ by Kruskal-Wallis test.

RESULTS

Plasma CRP: Dependence on tumor stage and grade

We first analyzed preoperative plasma CRP levels in all study patients with regard to tumor stage and grade. Analysis by Kruskal-Wallis test revealed that CRP plasma levels correlated tendentiously ($\chi^2 = 5.68$) ($P = 0.059$) with tumor stage and significantly ($\chi^2 = 6.52$) ($P < 0.05$) with grade, which is depicted in Figure 1. Partial correlation, corrected for age, gender, and tumor histology, confirmed a significant association between CRP values and tumor stage ($r = 0.3312$) ($P < 0.05$) as well as tumor grade ($r = 0.4327$) ($P < 0.01$).

CRP expression: Immunohistochemistry and mRNA quantification

To analyze a possible expression of CRP by RCC, we first studied the RCC cell line CaKi (kind gift from Dr. C. Frohn, Institute of Immunology and Transfusion Medicine, University of Luebeck, Luebeck, Germany) by means of quantitative real-time PCR. Native CaKi cells gave a clear amplification signal for detectable CRP mRNA, which was about five log ranks below the maximally stimulated Hep3B control cells (data not shown). Moreover, unstimulated CaKi cells showed a homogenous cytoplasmic CRP staining by monoclonal anti-CRP and APAAP technology (Fig. 2A and B).

In tumor specimens, we observed a perinuclear staining pattern for CRP by means of immunofluorescence staining that was either localized to single tumor cells of necrotic areas or to some tumor cells of the viable margins (Fig. 2C and D). Interestingly, CRP production sometimes appeared as a very bright homogenous staining pattern in clusters of tumor cells of tumor margins (Fig. 2E). In the surrounding renal tissue of RCC, CRP production was confined to the tubular epithelium, where staining followed the same pattern as observed for tumor cells: perinuclear staining of some epithelial cells, but bright cytoplasmic coloring in a few tubules as well (Fig. 2F). Due to the relatively poor quality of fresh-frozen tissue specimens, we were not able to define particular tubular segments responsible for these two different staining patterns.

We then investigated CRP mRNA expression in cDNA preparations from tumor-derived and surrounding tissues. Twenty six of 33 specimens (79%) derived from the tumor center, 30 of 36 (83%) from the tumor margin, but also 32 of 36 (89%) samples from adjacent healthy tissue were positive for CRP mRNA. Missing sample numbers to 40 are due to low RNA yields causing weak signals for the internal control HPRT that did not allow for definite conclusions about CRP expression. C_t values of the HPRT amplifications plots, which usually were in the range between 20 and 25, of greater than 30 served for exclusion from further analysis. Figure 3A gives representative examples of CRP and HPRT amplification plots for the three different kidney regions (pat. no. 41, see Table 1). In those samples, which could have been appropriately analyzed, a remarkably higher CRP mRNA expression was observed in tumor-derived tissues compared to the unaffected kidney poles. This difference was statistically significant between vital tumor margins and adjacent kidneys (Wilcoxon test, $P < 0.01$) (Fig. 3B). Nevertheless, the three different kidney regions correlated to each other in their degree of CRP mRNA expression with $r = 0.964$ for tumor center vs. margin, $r = 0.822$ for tumor center vs. adjacent kidney, and $r = 0.676$ for tumor margin vs. adjacent kidney (all $P < 0.001$). Furthermore, CRP mRNA expression levels increased significantly with tumor grade ($\chi^2 = 12.74$) ($P < 0.01$) for tumor centers; ($\chi^2 = 13.64$) ($P < 0.01$) for tumor margins; and ($\chi^2 = 6.73$) ($P < 0.05$) for adjacent kidney tissue, as given in Figure 3C, but not with regard to tumor stage

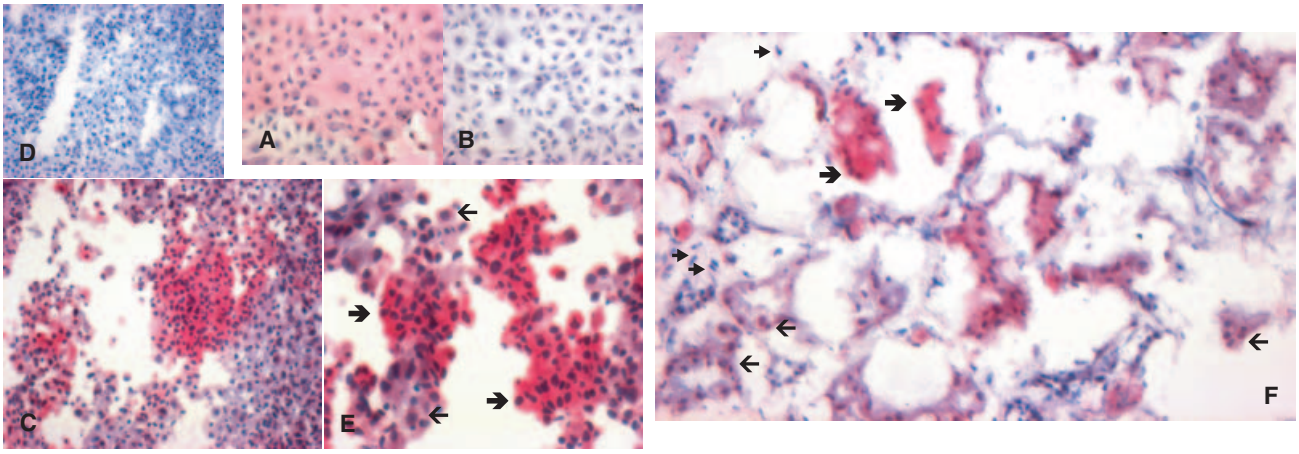

Fig. 2. Frozen sections of renal cell carcinoma (RCC) and surrounding renal tissue as well as Caki cells grown on chamber cultures slides immunostained for C-reactive protein (CRP) using APAAP methodology and naphthole/neofuchsin. Immunoreactivity was examined by monoclonal anti-CRP antibody (Sigma Chemical Co.). (*A*) Cytoplasmic, homogenous immunoreactivity for CRP in Caki cells (original magnification ×400). (*B*) Caki cells stained with an irrelevant isotype control antibody (original magnification ×400). (*C*) Perinuclear staining for CRP in some tumor cells of the viable margin besides tumor cell clusters with intense homogenous coloring (pattern 12, original magnification ×200). (*D*) Isotype control staining of the tumor margin (pattern 12, original magnification ×200). (*E*) Detailed view on CRP immunoreactivity in RCC tumor cells with perinuclear staining (←) or bright homogenous (→) coloring (pattern 12, original magnification ×400). (*F*) CRP immunoreactivity in adjacent renal tissue with perinuclear staining (←) of some tubular epithelial cells and a few more homogenously (→) stained tubules. (→) depicts interstitial cells negative for CRP (pattern 12, original magnification ×100).

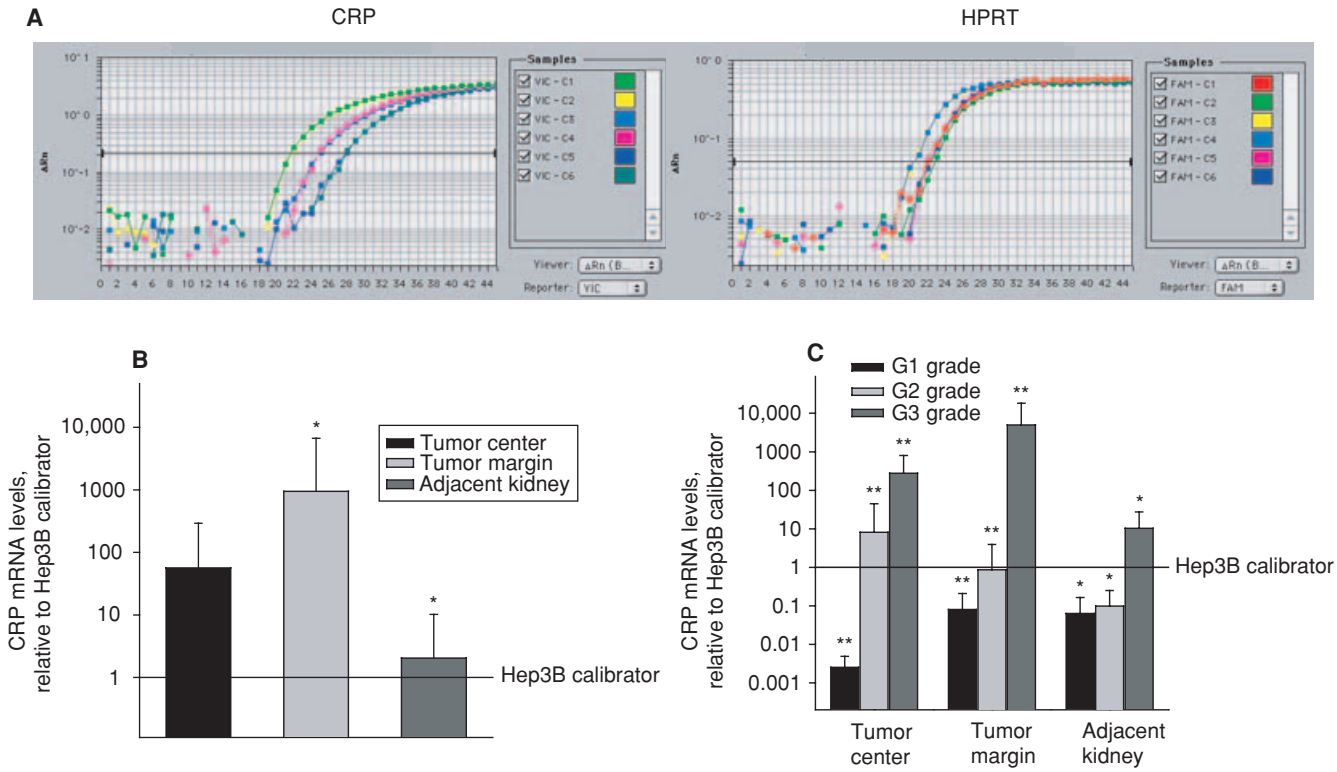

Fig. 3. Detection of C-reactive protein (CRP) mRNA by quantitative multiplex real-time TaqMan™ polymerase chain reaction (PCR). (*A*) Amplification plots for CRP (left panel) and for hypoxanthine phosphoribosyltransferase (HPRT) (right panel) mRNA detection in tumor center (C1 and C2), tumor margin (C3 and C4), and adjacent tissue (C5 and C6) of pattern 41. Y axis shows the normalized and baseline-corrected reporter signal ΔRn (VIC dye for CRP, FAM dye for HPRT detection), x axis shows the cycle numbers. Note the significantly lower ΔRn values for HPRT, which are due to limited primer/probe concentrations to avoid competition with concurring CRP mRNA amplification. (*B*) Mean CRP mRNA levels for all 40 renal cell carcinoma (RCC) in tumor centers, margins, and adjacent renal tissues. $^{*}P < 0.01$ by Wilcoxon test. (*C*) Mean CRP mRNA levels of the three different tumor regions with regard to tumor grading. Note the particularly strong positive correlation between tumor grade and CRP mRNA levels. $^{**}P < 0.01$ for tumor center as well as for tumor margin; $^{*}P < 0.05$ for adjacent kidney.

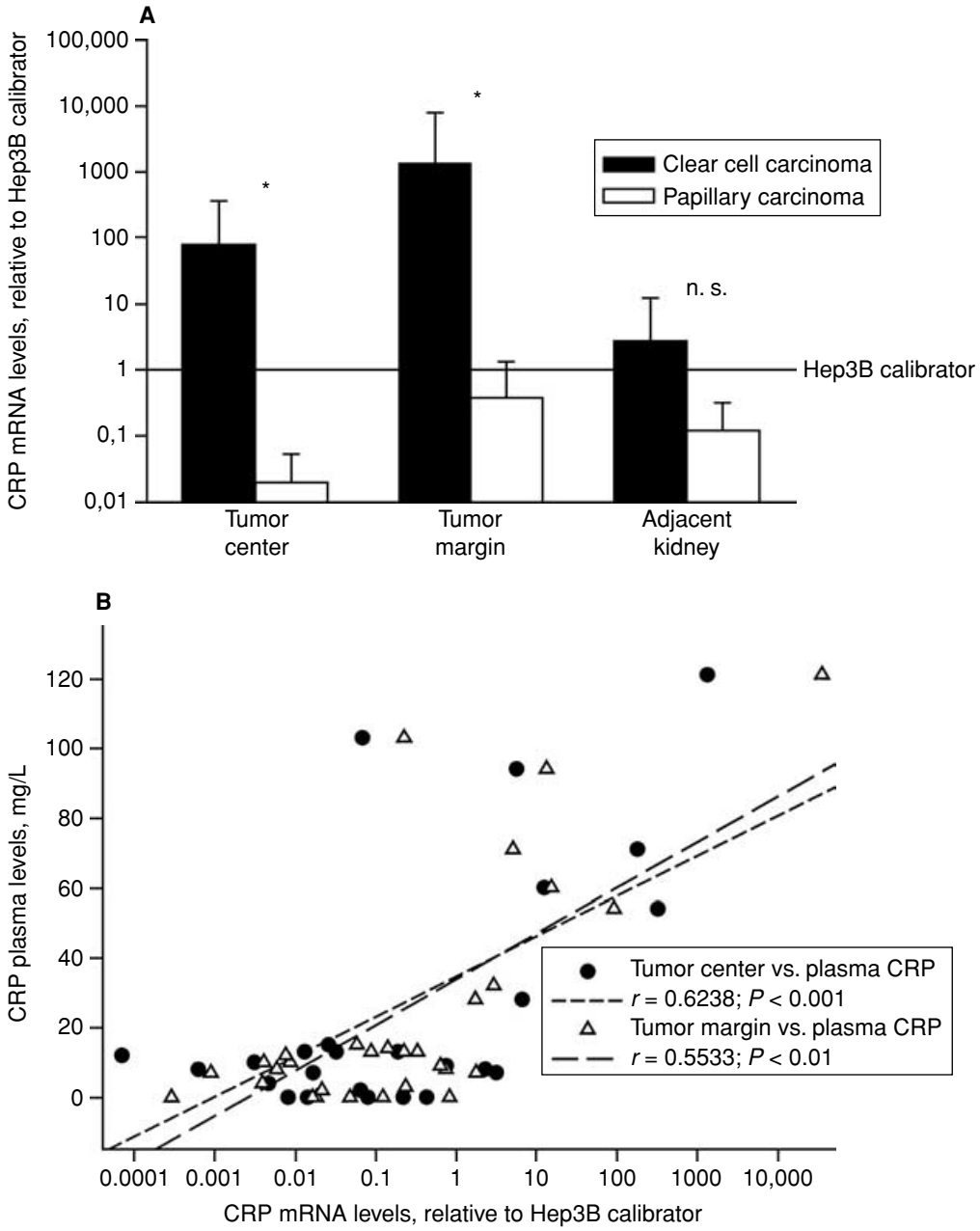

Fig. 4. Subtype-dependent C-reactive protein (CRP) mRNA expression of renal cell carcinoma (RCC) as well as correlation between intratumoral CRP mRNA expression and preoperative CRP plasma levels. (*A*) Analysis of CRP mRNA levels in the two main different RCC subtypes, clear cell and papillary, revealed significantly elevated levels in clear cell compared to papillary carcinoma. $^*P < 0.05$. n.s. is not significant. (*B*) CRP mRNA was quantified as described in detail in [3]. CRP mRNA of tumor centers and tumor margins are plotted against respective CRP plasma levels. The given correlations were highly significant, even when corrected for age, gender, and histology of the tumors.

(Kruskal-Wallis test, data not shown). Analysis of subtype-dependent CRP expression revealed significantly higher CRP mRNA levels in clear cell than in papillary carcinomas (Fig. 4A).

Correlation of plasma CRP with intratumor CRP and IL-6 mRNA expression

To correlate plasma CRP to intratumor CRP production, quantitative CRP mRNA data of the three different regions were compared to preoperative CRP plasma levels. This analysis revealed a highly significant positive correlation between the magnitude of CRP mRNA expression in all three kidney regions and CRP plasma levels, even when corrected for patients' age, gender, and histology of the tumor (shown in Fig. 4B for tumor centers and margins).

Finally, we investigated IL-6 expression of all tissue samples. Transcription of IL-6 mRNA was observed in

all tumors that also showed CRP synthesis by malignant cells and/or surrounding healthy tissue. Although the IL-6 expression levels in the different kidney regions were comparable to CRP mRNA levels, we did not find a significant correlation between IL-6 and CRP mRNA expression or between IL-6 expression and CRP plasma levels (data not shown).

DISCUSSION

In 2004, a total of 35,710 new cases of kidney cancer and renal pelvis cancer were expected in the United States [21]. At the time of diagnosis 54% of patients have organ-confined tumors, 21% locally advanced tumors, and 25% have metastatic tumors. Corresponding 5-year survival rates are 89%, 61%, and 9%, respectively. Standard therapy of organ-confined or locally advanced kidney cancer is operative removal of the tumor (total or partial nephrectomy, open operative, or laparoscopic approach). However, depending on tumor stage up to 50% of patients will develop metastases [22]. Standard therapy of metastatic kidney cancer includes systemic administration of cytokines like interferon (INF)-α and IL-2 either alone or in combination (also with chemotherapeutic agents like 5-fluorouracil or vinblastine) [23]. Unfortunately, median survival of these patients is 12 to 15 months and less than 10% survive for more than 5 years [24]. Rises in plasma CRP have repeatedly been proven to indicate tumor progression [12–14]. This study aimed to investigate a local origin of elevated plasma CRP in patients with RCC. We describe, for the first time, an autochthonous production of the acute phase protein CRP by RCC cells as well as by nontumorous renal tissue in vitro and in vivo. With respect to the specificity of CRP production in cancers, there is one study which described the immunohistochemical production of CRP by squamous cell carcinoma of the esophagus, however, not confirming this observation by transcriptional analysis of the CRP gene within the tumors [24]. Nevertheless, the authors concluded that intratumoral CRP expression along with high plasma CRP levels identified patients with unfavorable outcomes. We have started to extend our work on carcinomas of the prostate, the colon, and head, and neck squamous cell cancers, providing evidence for CRP gene transcription also in these entities (unpublished data). Another group showed an inducible expression of CRP by the human lung epithelial carcinoma cell line A549 [25], which again suggests that CRP expression by malignant cells is not restricted to RCC.

Like other studies, we observed remarkably high CRP plasma levels in some RCC patients, who nonetheless lacked clinical signs of infections (fever, chills, myalgia, coughing, or symptoms of urinary tract infection) and were in a satisfactory health state enabling radical nephrectomy. Thus, elevated CRP levels had to be attributed to the diagnosis of RCC. The positive correlation between the tumor stage (T_{1-3}) and CRP plasma levels is an interesting novel observation, which clearly demonstrates the relationship between tumor size and CRP plasma levels and therefore argues for a local origin of "tumor CRP." However, the mass of the tumor might not be the only factor influencing CRP plasma levels in RCC patients, and a contribution of liver-derived CRP to elevated plasma levels is likely, too, regarding considerably increased serum levels of IL-6 in these patients [15]. The extent of inflammation (either cellular infiltrates or autochthonous activation of proinflammatory signaling molecules) in the tumor may also influence CRP production of tumor cells. In fact, tumors with inflammatory reactions have been identified as particularly aggressive with a poor prognosis [26, 27]. CRP expression is induced by IL-6 and other factors, such as IL-1, complement components, and steroid hormones. The principally active transcription factors in the regulation of CRP expression in the liver bind to C/enhancer binding protein (EBP) and signal transducers and activators of transcription 3 (STAT3) response elements upstream of the promoter [28]. Furthermore, nuclear factor-kappaB (NF-κB) has recently been shown to participate in hepatic CRP induction and to act synergistically with C/EBP and STAT3 [29]. There is now compelling evidence that especially high-grade RCC display increased C/EBP-β as well as NF-κB activation causing an inflammatory, more aggressive phenotype [26, 27]. Activation of principal CRP inducers and enhancer proteins, therefore, may account for the significantly increased CRP expression in high-grade RCC with increased CRP plasma levels observed in this study. Increased activation of proinflammatory transcription factors causing CRP expression can be explained by autocrine secretion of IL-6 from tumor cells that may act via its receptor on RCC [30–32]. Although we detected IL-6 gene transcription in all samples positive for CRP mRNA, we were not able to correlate these findings to intratumoral CRP expression or to plasma CRP. IL-6 expression levels showed an extremely wide range over seven orders of magnitude. This strong diversity in IL-6 expression might result from p53 mutations in RCC, which have recently been shown to dramatically influence autocrine IL-6 production of RCC [32]. We also have to keep in mind that IL-6 is necessary, yet not sufficient for CRP induction, suggesting that other local factors within tumors regulate CRP expression as well. The observation that clear cell carcinoma express significantly more CRP than do papillary carcinoma is in line with a worse outcome for RCC patients with elevated CRP plasma levels and better survival rates for patients with papillary RCC [33]. Therefore, it is tempting to speculate that intratumoral expression of CRP indeed is an independent prognostic risk factor in RCC.

CONCLUSION

Our experiments suggest a local origin of "tumor CRP" in patients with RCC. Furthermore, the data justify monitoring of CRP levels in RCC patients after radical nephrectomy or other therapeutic interventions. Finally, these results contribute to the accumulating evidence that the acute phase reaction, or at least parts of it, does not always represent a systemic response pathway, but also takes place in local environments, such as inflamed [3, 34] or malignant kidneys.

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